PATENT SPECIFICATION (11) 1235379

1235379

NO DRAWINGS

(21) Application No. 25300/68 (22) Filed 27 May 1968
(31) Convention Application No. 856 (32) Filed 30 May 1967
(31) Convention Application No. 867 (32) Filed 16 Aug. 1967 in
(33) France (FR)
(45) Complete Specification published 16 June 1971
(51) International Classification A 61 k 27/14
(52) Index at acceptance
A5B 303 30X 30Y 365 36Y 382 38Y 39X 779

(54) PROCESS FOR EXTRACTING ANTHOCYANINS AND MEDICAMENTS CONTAINING SUCH COMPOUNDS

(71) We, STÉ DE RECHERCHES INDUSTRIELLES S. O. R. I., of 8, Rue Petiot, Dijon, France, a French Body Corporate, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a process for extracting anthocyanines from certain berries and fruits.

The term "anthocyanins" is intended to cover, when used throughout the specification, a compound of an aglycon with sugar. The identity of the aglycon varies according to the fruit or berries chosen. For example, the major aglycons found in backberries are cyanidol, delphinidol and malvidol.

It is already known that certain berries and fruits such as bilberries, elderberries, cherries, and black-currants contain large proportions of anthocyanins as pigments. They are used as colourants in the food industry, in confectionery as well as in the pharmaceutical industry in certain pharmaceutical preparations. They are obtained from a raw material which can consist of fresh fruits or frozen fruits preserved at a temperature of −20 degrees C. The prior art extraction process comprises, successively, the cutting up of the fruits by crushing and then their passage in a presser. The juice is squeezed out by renewed pressures; three pressings suffice generally to exhaust the fruit with maximum yield according to the present technique. The residue left from the pressing or "gennes" after these three successive pressings, is thrown away unless it can be used as a by-product.

The yield of juice can be considerably improved and the pressing operations can be substantially facilitated, according to a technique known widely in the fruit juice industry, by the adidtion of pectinases to the crushed fruits; these commercial enzymes have in fact the ability of hydrolyzing pectins and thereby to diminish the viscosity of the fruit juices.

This known technique however, does not make possible the recovery of the anthocyanin fraction which remains in the "gennes" which have been subjected to successive pressings. Additionally with respect to the purification of the material resulting from the pre-concentration of the juices, the present methods furnish a low yield and lend themselves poorly to an industrial scale of operation.

The invention thus seeks to provide a process for extracting anthocyanins which makes possible the extraction of anthocyanins from the "gennes" resulting from the first pressing; the preparation of unpurified extracts from anthocyanin-rich fruits; the purification of anthocyanins obtained from pre-concentrated extracts or unpurified extracts of fruits rich in these compounds and to make possible this purification on an industrial scale.

Certain therapetuic properties of anthocyanin-based extracts obtained by prior art processes are already known, in particular their vitamin P properties and those derived therefrom.

Experimentation has shown that the compounds and extracts obtained by this extraction process have substantially the same therapeutic properties as the compounds obtained by the prior art processes, but with a substantially increased activity and efficacity. Accordingly the present invention also has for its object the pharmaceutical utilization of the compounds and extracts prepared in accordance with the present process for treatment of certain conditions.

According to the present invention, there is provided a process for extracting anthocyanins from any one or any mixture of the following fruits, black-currants, bilberries, elderberries and cherries, comprising pressing and depectinising said fruit or fruits, separating the juice obtained thereby from the solid residues, macerating the solid residues in any one or any mixture of the following solvents, water, methanol, ethanol and butanol for a period of from four to twenty four hours, separating the residues and the liquid, adding said liquid to said juice and recovering the anthocyanins

[Price 25p]

from the mixture of liquid and juice by filtering the mixture and then concentrating the filtered liquid.

Also according to the present invention, there is provided a pharmaceutical composition containing anthocyanine prepared by the process defined in the last preceding paragraph together with a pharmaceutically acceptable inert carrier.

The solid residues maceration step may be effected in a stainless steel or plastics container with an equal weight of the solvent which may be acidified, if desired, with, for example, 1 or 2% by volume of hydrochloric acid.

The maceration operation may be repeated a second time and a third time; the maceration liquids are combined and mixed with the juice of the first pressings. Experience has shown that these three successive macerations achieve the extraction of the anthocyanins with a satisfactory yield.

The juice maceration liquids which have been combined are filtered preferably under a press, for example on cellulose discs and then the liquid obtained is concentrated, preferably, under partial vacuum 10^{-1} to $3\times10^{-}$ cm Hg at low temperature from 30° C to 50° C. for example, in a concentrating device with a centrifugal cone with a turbine or with a thin layer.

When methanol, ethanol or butanol or any mixture thereof is used as the extraction solvent, a trap device is added to the apparatus to ensure recycling of the solvent.

When a concentrate containing from 60 to 70% of its weight of dry material has been obtained by such evaporation, an unpurified extract can be obtained by complete evaporation under vacuum at a low temperature, that is, from 72° to 154° C in a device such as an atomizer or in a thin layer evaporator. Total desiccation of the extract can be effected, if desired, in the presence of an inert absorbent such as cellulose, talc, kaolin, kieselguhr, silica or calcium phosphate which is able to absorb some 70 to 80% of its weight of unpurified extract.

Such an unpurified extract, whether or not absorbed on an inert powder, forms one of the medicaments according to the present invention.

In the case of black-currants treated as above indicated by successive aqueous maccrations, the composition of the unpurified extract may be within the following ranges, by weight:

—27 to 30% of anthocyanins,

—traces of aglycon,

—30 to 35% of monosaccharides, mainly glucose, saccharose, and fructose,

—7 to 13% of mineral ions, in particular of potassium, magnesium, sodium, calcium, chlorides, oligo-elements such as iron, copper and cobalt,

—traces of pectins,

—organic ions, in particular the citrate, malate and lactate ions representing an organic acidity of 20 to 25%, expressed in terms of citric acid.

The liquid pre-concentrated to 60 to 70% by weight dry material in a concentrating apparatus as indicated above can be purified by one of the two following processes:

The pre-concentrated liquid and juice may be purified by means of ion exchange resins to form anthocyanin cations. These cations are selectively fixed by the anionic resins. The recovery of the anthocyanins takes place in strongly acid medium, in formic or hydrochloric acid for example.

In the second of these processes, there is produced a complex of an anthocyanins and neutral aluminium oxide. The aluminium oxide selectively fixes the anthocyanins and makes possible the elimination by washing of, in particular, the monosaccharides and organic acids. The anthocyanins can be obtained, for example, in the form of hydrochlorides if the dissociation of the complex is effected by an aqueous or alcoholic solution acidified by hydrochloric acid.

On an industrial scale, it is possible to use either of the above after chromatography, the liquid resulting from the preconcentrations constituting the descending moving phase.

The separation of the sugars, from one part of the mineral or organic ions is achieved by washing with slightly acidified water (0.5 to 1.5% of hydrochloric acid by volume for example); then the anthocyanins are eluated from one or the other column by a strongly acidified aqueous or alcoholic solution containing for example 20 to 30% by hydrochloric acid by volume. The eluation liquids are then evacuated under vacuum at a low temperature.

Again referring to the purifying of the liquid resulting from preconcentration, instead of using column, chromatography it is also possible to fix anthocyanins on one or the other of the two supports, that is to say the ion exchange resins or the neutralized aluminium oxide, by mixing this support with said liquid, the washings and the eluation are then carried out by successive decantations.

The purified extracts thus obtained contain substantially of from 70 to 75% by weight of anthocyanins and traces of aglycons retain a certain number of metallic cations in the form of complexes. The hydroxyl function is related to phenolic groups of the aglycon part of anthocyanins. These functions are able to form a chelate with metallic ions. A substantial part of these complexes is constituted of ferric ions present to the extent of 1.5 mg of iron per gramme of anthocyanins.

Such purified extracts are new products within the scope of the invention; they can constitute the base for medicaments intended primarily for parenteral use. The purified or

unpurified extracts can also have non-pharmaceutical applications.

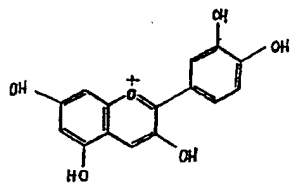
The expression "unpurified extract" means the powder obtained from drying the mixed extractive liquids obtained from fruits and "gennes". The expression "purified extract" means the powder obtained from the aluminium oxide or ion exchange resins eluates after chromatography.

These extracts are soluble in water and in slightly acidified methanol and ethanol. They give a violet red colouring to these solvents which varies toward blue when their pH increases; in the case of black-currants, the solution obtained is violet-red.

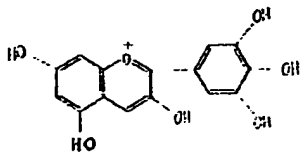
In the case of the black-currant extract, spectrophotometric examination shows two absorptions maxima which move according to the pH and whose wave length is of 278 and 520 nano meters in water with 1% hydrochloric acid by volume.

Lead acetate, picric acid and ammoniacal calcium chloride precipitate anthocyanins from their solutions.

By boiling in hydrochloric acid medium, the extract is hydrolized into anthocyanins which can be identified as cyanin cations



and delphinidin cations.



Use of chromatography with substantial quantities of extracts reveals the presence of a third aglycon which in all probability is malvidol. The monosaccharide fraction is identifiable as rhamnose and glucose.

The lethal doses at 50% (LD_{50}) of black-currant extracts have been determined intravenously and orally in Swiss mice of average weight of 20 g. The LD_{50} orally was greater than 25 g/kg. of animal weight, while the LD_{50} intravenously was between 1500 and 1600 mg./kg. for the unpurified and purified extracts.

Tests made on Albino rabbits has shown that the sub-chronic administration of 500 mg/kg. for a month did not produce any untoward side effects.

Pharmacological tests were made in order to determine the effect of these extracts on capillary permeability and resistance and on the circulatory dynamics.

These tests relates on the one hand to treatments with the unpurified extract and on the other hand with the purified extract, of black-currants (obtained one and the other as previously indicated) and as a comparison, treatments with rutin which is considered generally as having a good vitamin factor and which is currently used therapeutically.

Effect on capillary resistance

The measure of the depression applied for 10 seconds which is necessary to cause the appearance of red spots was effected in the normal rabbit or in rabbits fed with a vitamin deficient diet:

I) Tests on normal rabbits

The test is carried out in the sternal area which has been previously shaven off, a first time before initiating the treatment and then at the end of the treatment.

By way of examples, the average increase of the depression necessary for the appearance of the red spots were noted as follows:

—for 10 rabbits receiving for 15 days 100 mg/kg. orally of unpurified black-currant extract: average increase 37 mm of Hg.

—50 mg/kg. of rutin under the same conditions only occasion an increase of 15 mm of Hg.

—for 10 rabbits receiving for 15 days 50 mg/kg. orally of unpurified black-currant extract, an average increase: 25 mm of Hg.

—50 mg/kg of rutin under the same conditions only caused an increase of 16 mm of Hg.

—for 10 rabbits receiving for 10 days 20 mg/kg. orally of purified black-current extract, average increase: 35 mm of Hg.

—50 mg/kg. of rutin under the same conditions only caused an increase of 10 mm of Hg.

2) Experiments on vitamin deficient rabbits

For these animals, a first measurement was effected before initiating the diet consisting of bran and of cooked rice to which was added vitamins A, D, E and vitamins of the B group, a new measurement was effected after one month of this diet, before initiating the treatment, a third measure being made at the end of the treatment. The deficient diet is followed for the entire test.

By way of examples, the average increases of the depression necessary to cause the red marks were noted.

—for 10 rabbits receiving for four days 100 mg/kg. orally of unpurified black-currant extract, average increase between the second and third measurement: 35 mm of Hg.

—50 mg/kg. of rutin under the same conditions only caused an increase of 15 mm of Hg.

—for 10 rabbits receiving for 1 day 20 mg/kg. intraveneously of purified black-currant extract, average increase between the second and the third measurement: 20 mm of Hg.

—20 mg/kg. of rutin under the same conditions only caused an increase of 10 mm of Hg.

Activity on capillary permeability:

The measurement consists in checking the time for the appearance of an epidermal colouring under the effect of an intradermal injection of histamine hydrochloride. After the animals have received an intravenous injection of a colouring material such as Evan's Blue.

This measurement was made in the 150 gramme Albino rat and in the normal and deficient rabbit under the same conditions as in the previous test.

I) Tests on rats

The test is made in the dorso-lumbar area which has been previously shaven; the treatment consists in an intra-peritonial injection either of the unpurified or of the purified extract or of rutin.

By way of example, the average increases for the time required for the appearance of the coloured spot were the following:

—for an injection with unpurified black-currant extract at 200 mg/kg:

1 and a half hours after this invention: 44% increase

5 hours after this invention: 24% increase

—for an injection with purified black-currant extract at 20 mg/kg:

1 and a half hours after this injection: 32% increase.

—50 mg/kg of rutin under the same conditions did not cause any decrease in capillary permeability.

2) Tests on normal rabbits

The test is made in the previously shaven sternal area; the protocol is the same as that for the previous test, the treatment consists in a daily oral administration either of the purified or of the unpurified extract or of rutin.

By way of example the increase in the time for the appearance of the coloured spots was the following:

—for 10 rabbits receiving for 15 days 100 mg/kg. of unpurified black-currant extract: 36% increase,

—for 10 rabbits receiving for 15 days 50 mg/kg. of unpurified black-currant extract: 22% increase

—for 10 rabbits receiving for 10 days 20 mg./kg. of purified black-currant extract: 34% increase.

—for 10 rabbits examined as a control and which had received 50 mg/kg. of rutin under the same conditions, there was noted no modification in the capillary permeability.

3) Tests on the vitamin-deficient rabbit

The test is carried out as before and the technique consists in measuring the differences in the times needed for the appearance of the coloured spot, before the deficiency, after a month under deficient diet, and after the treatment associated with the deficient diet.

By way of example there was noted the following:

—for 10 rabbits receiving for 4 days 100 mg/kg. orally of unpurified black-currant extract: average increase between the first and third measurement: 140%.

—50 mg/kg. of rutin administered under the same conditions caused an increase of 60% in the time lag for the appearance of the colouration.

—for 10 rabbits receiving for one day 20 mg/kg. intravenously of purified black-currant extract: average increase between the second and the third measurement: 80%.

—20 mg/kg. of rutin administered under the same conditions caused an increase of 27% in the time lag for the appearance of the colouration.

The conclusion of these different tests is that it is remarkable to have obtained systematically an efficacity which is greatly superior with the purified or unpurified anthocyanin extracts obtained by the present invention relatively to rutin which is considered generally as having a good vitamin P factor and which is currently used therapeutically.

In effect if the values obtained during tests with the respective amounts of anthocyanins contained in the purified or unpurified extract obtained according to one of the ways described above with the rutin administered, it is noted that the tests show that the anthocyanins thus obtained by the present invention are from 3 to 6 times more active than rutin for equal doses.

Effects on circulatory dynamics

The test consists in checking the effect on diuresis and arterial pressure resulting from a treatment with purified or unpurified extracts in the rat rendered experimentally hypertensive by tying for example a renal artery.

For animals showing an average increase in their arterial pressure of 40%, a decrease of diureses of 35%, a treatment consisting in the daily oral administration of 50 mg/kg. of unpurified black-currant extract leads to a lowering of hypertension to 27% and to the re-establishment of a substantially normal diuresis for the average of the animals.

The general effects on the circulation of the anthocyanins in conformity with the invention made possible their use for a great number of human conditions, in particular:

—the venous circulatory troubles, valve strains, thrombosis, sclerosis and varicose veins,

—general circulatory troubles following arteriosclerosis, arterial hypertension, diabetes, thrombosis,

—Raynaud's disease,

—complaints arising from capillary permeability and fragility and from purpuras of various origins,

—secondary manifestations resulting from treatments with anti-coagulants,

—allergic phenomena,

—heamorrhoid conditions.

The combined actions of citrates and of anthocyanins in the proportions of the obtained extract causes an increase in the vascular proportions of the anthocyanins, in particular with respect to their long acting effect.

Thus rats receiving a daily oral dose of 20 mg/kg of unpurified extract from the eighteenth to the twenty-fourth month have an arterial pressure at 90 mm of Hg which is less than that of the animals treated under the same conditions but receiving 6 mg/kg of pure anthocyanins and whose arterial pressure is of 100—110 mm of Hg.

On the other hand both treatments have an anti-hypertensive effect presumably linked to the trophic vascular effect which opposes itself to the ageing of the animals, the control rats having an average arterial pressure of 120 mm of Hg.

Case Histories

Case 1

Mr. BER . . . aged 64

Examination of the bottom of the eye shows considerable sclerosis of the arterial vascular network; the patient is hypertensive; the least shock causes the appearance of ecchymosis; the capillary resistance tested by the capillo-dynamometer of Lavollay is abnormally low.

After the administration of two capsules per day containing 50 mg of unpurified black-currant extract for 15 days, the capillary fragility diminishes substantially (increase of the threshold of depression=20 mm of Hg measured by capillo-dynamometer); the the ecchymotic tendancy disappears.

Case 2

Mrs. TIR . . . aged 45

This patient had to remain standing for long periods of time because of the nature of her work; she complains of venous stasis of the lower limbs, and external varicose veins are already apparent.

Treatment with capsules containing 50 mg. of unpurified black-currant extract was carried out at the rate of one capsule per day for a month.

The syptoms of venous statis disappear upon auscultation showing partial recovery of elasticity in the venous walls; the heavy legged feeling is only intermittent and the oscillometric indicia of the calf returns to normal.

Case 3

Mr. MAR . . . aged 57

After a cerebral thrombosis going back to about 6 months, a chronic treatment with dicoumarol was initiated; it resulted after this time in haemorrhagic and ecchymotic tendencies.

Treatment with capsules containing 10 mg. of purified black-currant extract at the rate of one capsule per day for a week caused the capillary fragility complaint to disappear.

Case 4

Mr. PER . . . aged 48

This patient has been a diabetic for a long time and is treated with insulin and with sulfonamides and shows signs of vascular degeneration, in particular upon examination of the bottom of the eye with clinical manifestations of arteritis.

He was given capsules containing 10 mg. of purified black-currant extract at the rate of one capsule per day for a month.

There was noted a distinct improvement upon examination of the bottom of the eye; the arterial pressure stabilizes itself at 12—14; the signs of vaso-constriction recede.

The purified or unpurified extracts according to the invention can be administered:

—orally in the form of tablets, lozenges, gels, capsules, drinkable ampoules, drops, containing 10, 20, 50 or 100 mg. of purified or unpurified extracts per dosage unit.

—or rectally in the form of suppositories or capsules containing 10, 20, 50, 100 or 200 mg. of purified or unpurified extract.

—or externally in the form of pomades, creams, lotions, in which the raw or purified extract is present in a concentration ranging from 1 to 5% in an excipient which makes it possible to penetrate the epidermal barrier.

WHAT WE CLAIM IS:—

1. A process for extracting anthocyanins from any one or any mixture of the following fruits, black-currants, bilberries, elderberries, and cherries, comprising pressing and depectinising said fruit or fruits, separating the juice obtained thereby from the solid residues, macerating the solid residues in any one or any mixture of the following solvents, water, methanol, ethanol and butanol, for a period of from four to twenty four hours, separating the residues and the liquid, adding said liquid to said juice and recovering the anthocyanins from the mixture of liquid and juice by filtering the mixture and then concentrating the filtering liquid.

2. Process according to claim 1, wherein said separated solid residue following maceration is subjected to at least one additional maceration.

3. Process according to claim 1, wherein said liquid and juice is pre-concentrated under a partial vacuum at a temperature from 30° to 50° C until from 60 to 70% by weight thereof consists of dry material.

4. Process according to claim 3, wherein when the macerating solvent is methanol, ethanol, or butanol or any mixture of these solvents, the vapours are recovered and recycle.

5. Process according to claim 3, wherein the pre-concentrated material is completely dried under vacuum at a temperature from 72° to 154° C in the presence of an absorbent, inert powder.

6. Process according to claim 5, wherein said inert powder is any one or any mixture of cellulose, talc, kaolin, kieselguhr, silica or calcium phosphate.

7. Process according to claim 1, wherein the solvents used for maceration are acidified.

8. Process according to claim 3, wherein said pre-concentrated liquid and juiece is purified by means of ion exchange resins to form anthocyanin cations and said ion exchange resins are then eluted in an acid medium.

9. Process according to claim 3, wherein said pre-concentrated liquid and juice is purified chromatographically on a moving column, said pre-concentrated liquid and juice is cending movable phase on said column.

10. Process according to claim 3 wherein said pre-concentrated extract is purified by the formation of a neutralized aluminium oxide-anthocyanins complex which is then eluted in an acid medium.

11. Process according to claim 7, wherein said anthocyanins are fixed on said ion exchange resins by mixing therewith.

12. Process according to claim 7, wherein the acidified liquids obtained after maceration are evaporated to dryness under a reduced pressure from 10^{-1} to 3×10^{-1} cm Hg and at a temperature from 30° C to 50° C.

13. Process according to claim 8, wherein said pre-concentrated liquid is concentrated to dryness under a reduced pressure from 10^{-1} to 3×10^{-1} cm Hg and at a temperature from 30° C to 50° C.

14. A pharmaceutical composition containing anthocyanins prepared by a process as claimed in any preceding claim together with a pharmaceutically acceptable inert carrier.

MARKS & CLERK,
Agents for the Applicants.

Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1971.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.